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ECBC-TR-1141

DEVELOPMENT OF A CONTACT PERMEATION TEST FIXTURE AND METHOD

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RESEARCH AND TECHNOLOGY DIRECTORATE

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

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14. ABSTRACT:
A new fixture and method were developed to quantify the cumulative permeated mass of contaminants through personal protective equipment (PPE). Most PPE testing consists of liquid contamination of a swatch and detection by vapor collection. However, vapor analysis may not be the best detection method for all contaminants. Some contaminants, including VX, are contact hazards due to their low volatility. This property, coupled with the fact that PPE can be in direct contact with the skin, indicates the need for a quantitative contact test method. Comparison tests were conducted with VX on a standardized latex material in vapor and contact configurations using analytical permeation methods and toxicological percutaneous testing. For both the permeation and the toxicological configurations, approximately 20-fold more contaminant was measured using contact rather than vapor testing. This indicates that vapor testing could underestimate the amount of agent present in a contact scenario. As new PPE articles are developed, it is important to test each material in a manner consistent with its expected use to better understand the protection performance.

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PREFACE

The work described in this report was authorized under Defense Threat Reduction Agency (DTRA) Joint Science and Technology Office (JSTO) Project No. BA06DET504 and Joint Program Manager – Contamination Avoidance, Product Director Test Equipment, Strategy, and Support (JPM-CA, PDTESS) Project No. 21-0-2040-0000-6N-6N66-62262255200-S19130: 0NGH24. The work was started in September 2008 and completed in February 2013.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. Manufacturer names and model numbers are provided for completeness. This technical report may not be cited for purposes of advertisement.

In conducting the research described in this report, investigators adhered to the requirements in *Guide for the Care and Use of Laboratory Animals* (8th ed.; National Research Council: Washington, DC, 2011). This test was also performed in accordance with the requirements of AR 40-33, *The Care and Use of Laboratory Animals in DoD Programs* (Department of the Army: Washington, DC, 2005) and in compliance with good laboratory practices (Code of Federal Regulations; Title 40: *Protecting the Environment*, Part 792: Good Laboratory Practice Standards; U.S. Environmental Protection Agency: Washington, DC, 2005). This report compiles results from several previous studies. Final approval for these studies was granted by the U.S. Army Edgewood Chemical Biological Center (ECBC) Laboratory Institutional Animal Care and Use Committee. Protocol 08-400 was approved on 14 May 2008, and protocol 09-415 was approved on 1 June 2009.

This report has been approved for public release.

Acknowledgments

A program cannot be successfully completed without the contributions of a good team of people. The following individuals are acknowledged for their assistance with the execution of this program:

- Dr. Ngai Wong (DTRA JSTO) for funding and support of this program.
- Steven Harlacker and Gail Soubie (JPM-CA PDTESS) for funding and support of this program.
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- Michael Sheely (ECBC) for discussions regarding analytical permeation methodology.
- Jennifer Horsmon and Dr. Robert Mioduszewski (ECBC) for review and editing of the report.

Toxicology Quality Assurance Statement

This study, conducted as described in Protocol 08-400 and Protocol 09-415, was examined for compliance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency in 40 CFR Part 792. The report of the study is titled "Development of a Contact Permeation Test Fixture and Method". The dates of all inspections and the dates the results of those inspections were reported to the Study Director and management were as follows:

Phase Inspected	Date	Reported
Data and Final Report	13 Nov 2009	13 Nov 2009
Data and Final Report	26 Feb 2013	26 Feb 2013

To the best of my knowledge, the methods described were the methods followed during the study. The report was determined to be an accurate reflection of the raw data obtained.

JACQUELINE A. SCOTTO
Quality Assurance Auditor
Toxicology and Obscurants Division
Research and Technology Directorate

Analytical Permeation Quality Assurance Statement

All analytical permeation data produced for this project was reviewed and was found to have met the data quality objectives satisfactorily. This review was performed in accordance with the Permeation and Analytical Solutions Team Quality System documentation and the guidance found in the ISO 17025 standard.

All permeation and analytical testing was performed March through May 2011.

CATHERINE M. STERN
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DEVELOPMENT OF A CONTACT PERMEATION TEST FIXTURE AND METHOD

1. INTRODUCTION

1.1 Background

Permeation testing is a standard application for evaluating the diffusion of chemicals through a barrier. This is an important factor in choosing personal protective equipment (PPE) for laboratory personnel, industrial plant operators, farm workers, and emergency responders who interact with chemicals.[1]

Most permeation testing relies upon vapor collection[2] or solubility in water or other liquid that will not interact with the test material.[3] However, the use of contact sorbent pads is also well established for quantifying uptake of contaminants from environmental settings.[4] In many of these studies, the use of these pads has been benchmarked for organophosphorous pesticide exposures.[5–8] Low-volatility organophosphorous pesticides often serve as simulants for chemical warfare compounds, including VX.

Similarly, a silicone pad was used as a sorbent medium and compared to liquid medium collection and solvent splash methods.[9] This approach was also used to examine a range of protective glove materials.[10] Such efforts were expanded to examine and compare a large range of sorbent materials, pressures, and contaminants for contact testing.[11]

A recent permeation program was initiated to examine the performance of PPE against low-volatility contaminants such as VX. Given the low volatility of the compounds of interest, the standard vapor detection methods were deemed insufficient without modification. Furthermore, VX has low water solubility, and organic solvents are not compatible with the test materials; thus, use of the ASTM method with a solvent collection was not appropriate. To address this need, a hybrid method was devised to enable quantification of breakthrough in a contact scenario with environmental control. In this method, a sorbent pad was placed under the material swatch within a standard test cell. At the chosen time, the swatch was removed from the cell and extracted. The extractant was analyzed to quantify breakthrough. Limitations for this method included the variable contact level between the swatch and the sorbent pad. This was exacerbated with non-flat swatches taken from fingers of gloves or folded portions of protective suits. The non-intimate contact was manifested in large variances of permeated masses, which exceeded ±80%.[12] Furthermore, there was no method to apply relevant forces of contact. The use of the test cell made this system cumbersome, and each swatch was limited to a single time point. Therefore, a new fixture and method were needed to improve accuracy and throughput.

This report describes the development of an improved fixture and method for measuring the permeation of low-volatility chemicals through protective equipment. The primary goal was to develop a fixture and method that would accurately describe the performance of PPE materials. Additional goals were to meet or exceed current test requirements, increase throughput, and improve the variance over the standard test methods. Quantitative analysis comparisons between various permeation test methods are provided.

1.2 Development of a Contact-Based Permeation Test Method

During hybrid method testing, it was observed that the swatch was not always in good contact with the sorbent pad. This was hypothesized as a primary driving factor for the variance in permeation results. To overcome this, a new contact method was developed whereby the swatch and sorbent pad were forced to be in contact by means of a stainless steel weight, as shown in Figure 1.

The fixture consisted of a polycarbonate Petri dish, a polytetrafluoroethylene (PTFE) liner, a sorbent pad, a contaminated swatch, a smaller-diameter PTFE liner, and a stainless steel weight capable of delivering 1 psi to a 1 in.^{2} region. These items were contained within an inverted 8 oz glass jar to allow for containment and safety in handling. With the exception of the stainless steel weight, the entire fixture was disposable, which reduced the potential for cross-contamination. Temperature control was achieved with an incubator (VWR International; Radnor, PA) that had been modified with sliding shelves to facilitate access to individual jars. The simplified design allowed for a greater throughput, improved variance in results, and a lower investment of specialized infrastructure. A patent is pending on this fixture and method.

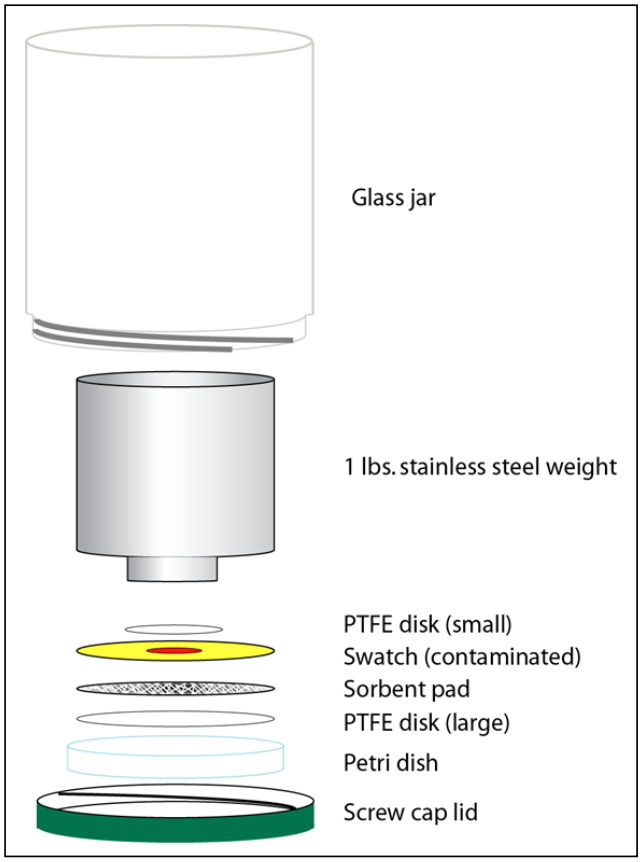

Figure 1. Schematic of the new contact test fixture (patent pending).

The 1 psi pressure was chosen for several reasons. First, the forces were relevant to contacting a contaminated surface or grasping a contaminated object. This was documented as a "heavy" touch for dermal transfer to the hands during residential pesticide applications ranging from 0.1 to 1.0 psi.[13] The grip pressure for using hand-tools, including hammers, has been documented as 1.5 psi.[11] This same study measured the effects of pressures from 0.1 to 2.8 psi on permeation. The applied pressure of 1 psi was well within the range of expected pressures for manual operations and was also mandated by an expulsion test in standard test procedures.[2]

2. EXPERIMENTAL PROCEDURES

Caution: The handling of chemical warfare agents should only be performed by trained personnel at an approved facility using applicable safety, security, and surety precautions.

All animals were handled in accordance with (IAW) animal use guidelines and a sanctioned protocol under the auspices of the U.S. Army ECBC Institutional Animal Care and Use Committee (IACUC).[14–16]

For all experiments, the material substrate was a natural latex swatch with a thickness of 10 mil (0.010 in.; McMaster-Carr; Robbinsville, NJ). The temperature was equilibrated to 37.2 °C and held to within 1 °C. Flow rates, where applicable, were adjusted to 300 mL/min. The permeation time was 4 h.

2.1 Toxicological Percutaneous Testing

The evaluation of percutaneous testing included multiple independent endpoints to determine the permeation of VX through a positive-control swatch material. These endpoints included toxic signs, periodic monitoring of acetylcholinesterase (AChE) activity levels, and recovery assays of VX from skin tissue and blood plasma.

New Zealand White male rabbits were procured from Millbrook Breeding Labs (Amherst, MA) and held in acclimation for 1 week before use. Rabbits weighing 2.3–3.2 kg were prepared for percutaneous testing by clipping the fur from an area of approximately 150 cm^2 on each animal's back. This process was performed the day before testing. The skin regions for all tests were free of any imperfections that could influence contaminant permeation.

On the morning of the test, each rabbit was weighed on a calibrated Toledo balance to the nearest 0.01 kg then placed into an aluminum stanchion. The stanchion was open on the top, but the sides and bottom were solid. The stanchioned rabbits were placed into a chemical agent fume hood. An area the size of the material panel was demarcated on each animal's back with black marker. Before dosing was started, a baseline AChE blood sample was acquired from the marginal ear vein of each animal.

Permeation materials were prepared by affixing 2 × 3 in. swatches to a metal template holder with tape. A raised edge on the template allowed the contaminated region to be covered yet remain untouched by 6 mil polyethylene film. This covering was used as a safety precaution. For offset testing, an additional 1 cm spacer was inserted to elevate the swatch from the skin.

At the time of dosing, the rabbit skin was swabbed with saline (Vetco; Melville, NY) to simulate a sweaty skin condition before the swatch was attached to the animal. The working area of the swatch was then contaminated with eight 1 µL drops of VX. The direct contact and offset configurations immediately after contamination are shown in Figures 2 and 3, respectively. Next, a polyethylene film was placed over the template holder and attached with tape. The entire device was secured with strip of flexible, self-adhesive veterinary wrap (Andover Healthcare; Salisbury, MA).

Animals were continuously monitored for toxic signs, including fasciculations, free-flowing salivation, peripheral nervous system (PNS) tremors, pinpoint miosis, postural changes, tonic/clonic seizures, full-body tremors, gasping, cyanosis, collapse, prostration, and death.

Serial blood samples were collected from the marginal ear vein at set times for evaluating AChE activity. The time points for sample collection were varied for each group of animals. Earlier time points were included for the positive-control animals because the AChE level was expected to be affected sooner. AChE analysis is described in Section 2.1.2.

The swatch remained on the rabbit's back for 4 h or until death. During the offset test protocol, animals that survived were euthanized at 4 h. During the direct-contact test protocol, animals that survived were euthanized at 48 h. Euthanization was performed by injection of 80 mg/kg of Fatal-Plus solution (Vortech Pharmaceuticals; Dearborn, MI) into the marginal ear vein. The negative-control animals were the only rabbits to be returned to their cages after the test, and were decontaminated at 24 h with a 0.5% hypochlorite solution before being removed from engineering controls.

Next, the skin was excised and placed in a plastic tube for analysis. Skin samples were frozen in liquid nitrogen. Once the skin had been excised, 5 mL of blood was acquired for recovery analysis using a heart stick with a 10 mL syringe and an 18 gauge needle. Blood samples were refrigerated until they were delivered to the analytical group for analysis.

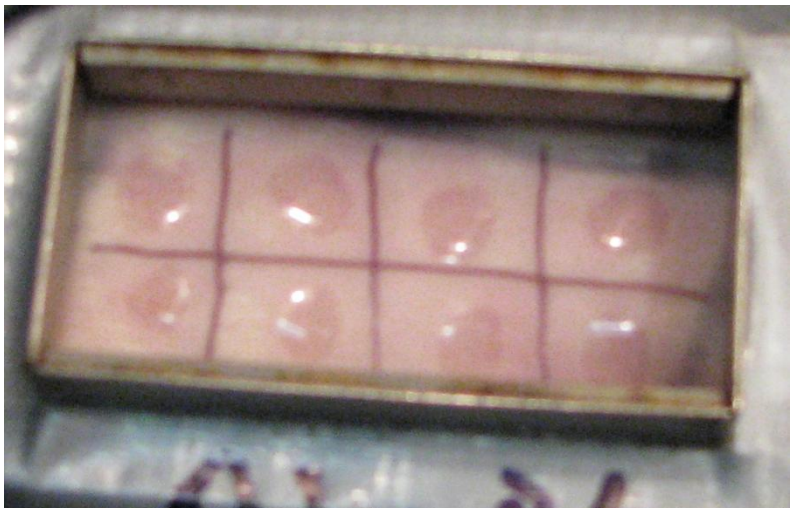

Figure 2. Contaminated latex in direct contact with skin, rabbit 71.

Figure 3. Contaminated latex offset from skin, rabbit 243.

2.1.1 Toxic Sign Evaluation

Animals were constantly observed for toxic signs from the time of exposure until 1700. A video system was used to record the time of death for animals overnight. A software program developed in-house was used to correlate elapsed time to particular toxic signs within six categories. The categories and associated toxic signs are noted in Table 1. It was difficult to observe localized fasciculations in the test rabbits because the panel occluded the exposure site. Furthermore, the process of removing a panel can induce localized fasciculations.

Table 1. Toxic Sign Categories and Associated Individual Signs

Category	Toxic Signs
Local	Localized fasciculations
Discrete	PNS tremors Pinpoint miosis Free-flowing salivation
Diffuse	Altered mental status Whole-body tremors Tonic/clonic seizures Postural changes Involuntary movements
Cardiorespiratory	Gasping Cyanosis
Moribund	Collapse Prostration
Death	Death

2.1.2 AChE Evaluation

A modified Ellman procedure was used to determine the AChE activity levels in the blood samples.[17] Unlike traditional procedures in which the measurement was based on the reaction of esterase with Ellman reagent, 5,5′-dithiobis-(2-nitrobenozic acid) (DTNB), to form a color compound, the modified assay was based on the molar absorptivity of 2-nitro-5-chlorobenzaldehyde (TNB^-) at 450 nm and 37 °C to the sodium dodecyl sulfate–hemoglobin reagent (SDS–HGB) complex at 536 nm. By using the respective extension coefficients of TNB^- and SDS–HGB, the values for AChE were obtained and normalized to the pretest value, with the 0 min sample set to 100%. The severity of cholinesterase depression was categorized into several levels that were determined by the lowest level of AChE activity, as shown in Table 2.

Table 2. AChE Severity Levels Compared with Activity Values

Severity of AChE Depression	Category Number	AChE Activity Value (%)
None	0	≥80
Mild	1	50–80
Moderate	2	25–50
Severe	3	<25

2.1.3 Tissue Analysis

Blood and tissue samples may be analyzed for VX as either a free compound or as regenerated G-agent analog (VX-G), which accounts for bound as well as free contaminant. For these studies, the regenerative method was used.[18, 19]

For VX-G determinations in blood plasma, a 0.4–0.5 g aliquot sample of the plasma was weighed in a tared microcentrifuge vial before the addition of 750 µL of pH 3.5 acetate buffer. The acetate buffer consisted of 0.01 M sodium acetate, 0.2 M glacial acetic acid, 300 µL of 6 M potassium fluoride, and 1 µL of deuterated VX-G internal standard. This mixture was vortexed for 20–30 s then centrifuged at 15,000 rpm for 5 min in a Micromax microcentrifuge (Thermo IEC; Needham Heights, MA) to yield a supernatant.[18]

For VX-G determinations in tissue samples, 0.2–0.5 g weighed aliquots of pulverized sample were further processed using the S-series focused acoustic energy system (Covaris; Woburn, MA). Next, 2 mL of pH 3.5 acetate buffer, consisting of 400 µL of 6 M potassium fluoride, 600 µL of 1 M hydrogen chloride, and 1 µL of the internal standard, was added to a borosilicate culture tube that contained the weighed tissue sample. The mixture was homogenized using the Adaptive Focused Acoustics process (Covaris), then centrifuged at 4400 rpm for 10 min in a Thermo IEC Centra-GP8R centrifuge to yield a supernatant.[19]

The resulting supernatants were loaded onto a previously conditioned 3 cc Oasis HLB extraction cartridge (Waters; Milford, MA). The solid-phase extraction (SPE) cartridge was conditioned by drawing through 1 mL of ethyl acetate, followed by 1 mL of 2-propanol and 1 mL of acetate buffer. The pellet from the centrifuged sample was resuspended with an additional 750 µL of acetate buffer and 300 µL of potassium fluoride, and was vortexed and centrifuged as before. Additional supernatant was added to the SPE cartridge, and the entire mixture was allowed to drain through it. The SPE cartridge was then dried under vacuum for 5 min. The VX-G and internal standard were eluted and collected with the addition of 1 mL of ethyl acetate under a gentle vacuum. This fraction was further dried with the addition of enough anhydrous sodium sulfate to allow it to freely flow in the vial. The dried sample was filtered through a 0.2 µm nylon Acrodisc syringe filter (Pall Corporation; Port Washington, NY) that had been prerinsed with 1 mL of ethyl acetate before it was transferred to a gas chromatography (GC) autosampler vial. Before analysis, the sample was evaporated under a nitrogen stream at room temperature to a 50 µL volume.

Analysis was performed on an Agilent Technologies (Wilmington, DE) model 6890 GC system interfaced to a Micromass Quattro micro GC, tandem-quadrupole mass spectrometer (MS; Waters). GC separations were achieved using a Restek (Bellefonte, PA) Rtx-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The carrier gas was helium at a 1 mL/min flow rate. An automatic liquid sampler (ALS) model 7683B autoinjector (Agilent Technologies) was used to inject 1.0–3.0 µL samples into a splitless injector port at 225 °C. The initial oven temperature of 35 °C was held for 2 min, ramped at 15 °C/min to 125 °C, then ramped again at 30 °C/min to 325 °C. Elution times for both VX-G and deuterated VX-G were typically 5 min. Samples were ionized using positive-ion chemical ionization with ammonia reagent gas.

The triple-quadrupole MS was operated in multiple-reaction monitoring (MRM) mode. For VX-G, the MRM program monitored the mass-to-charge ratio (*m/z)* transition from 144 to 99 for quantification. For the internal standard, the *m/z* transition from 149 to 100 was monitored. The MassLynx application software provided with the Quattro micro GC was used to process and analyze the data. This included the QuanLynx software, which provided automated peak detection, calibration, and quantification using an 11-point calibration curve with standards ranging from 1.0 ng/mL to 1.0 µg/mL of

VX-G and 250 ng/mL of internal standard. The detector response with respect to the relative concentration was linear with typical correlation coefficient values of 0.998. Separate quality control and matrix spike check standards were analyzed before each batch of samples. The practical quantification limit for most samples was 0.5 ng (500 ppt) of VX-G per gram of biological matrix, which was equal to the lowest standard in the calibration curve.

2.2 Analytical Permeation Testing

2.2.1 Vapor-Only Testing

Standard aluminum Aerosol-Vapor-Liquid Assessment Group (AVLAG) cells were loaded with a latex swatch and secured, as shown in Figure 4.[2] The permeated samples were collected in Tenax sorbent-loaded tubes. Each cell was loaded with 10 mg of VX dispensed as single 1 µL droplets. After 4 h, these tubes were removed for extraction with 10 mL of acetonitrile to measure the cumulative permeated mass. Analysis was conducted using gas chromatography with flame photometric detection (GC-FPD).

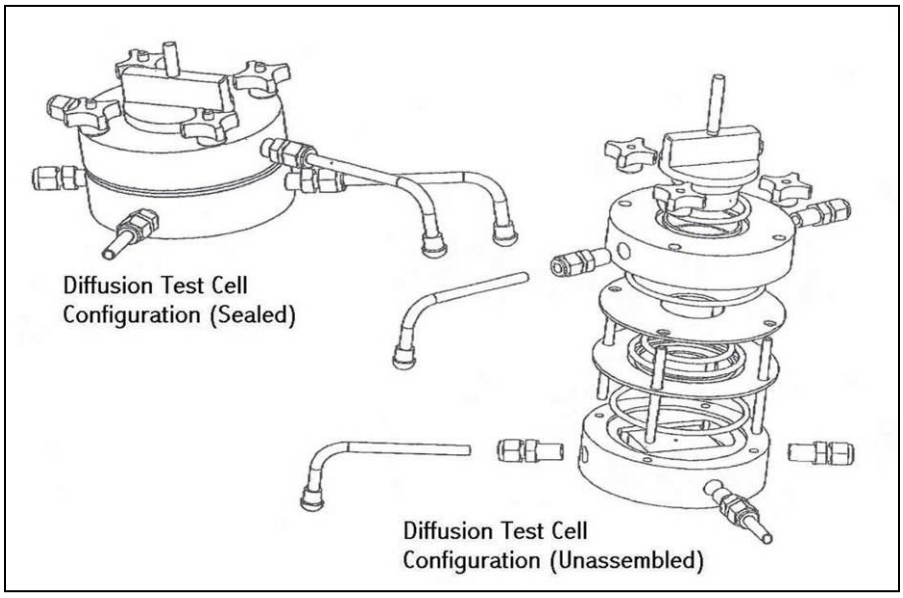

Figure 4. An AVALG cell.

2.2.2 Hybrid Testing

A sample package was created with a latex swatch atop of a sorbent pad (Empore solid-phase extraction disks, styrenedivinylbenzene [DVB], part SDB-XC; 3M; St. Paul, MN), that had been previously prepared IAW the manufacturer's instruction. These two materials were placed on a perforated stainless steel support screen, as shown in Figure 5. The permeated vapor samples were collected in Tenax sorbent-loaded tubes. A total of 10 mg of VX was loaded onto the cell in single, 1 µL droplets. After 4 h, the tubes were removed for extraction with 10 mL of acetonitrile to measure the cumulative permeated vapor mass. The AVLAG chamber was disassembled, and the sorbent pad and support screen were extracted in 20 mL of acetone for 30 min to measure the cumulative permeated contact mass. Analysis was conducted using GC-FPD.

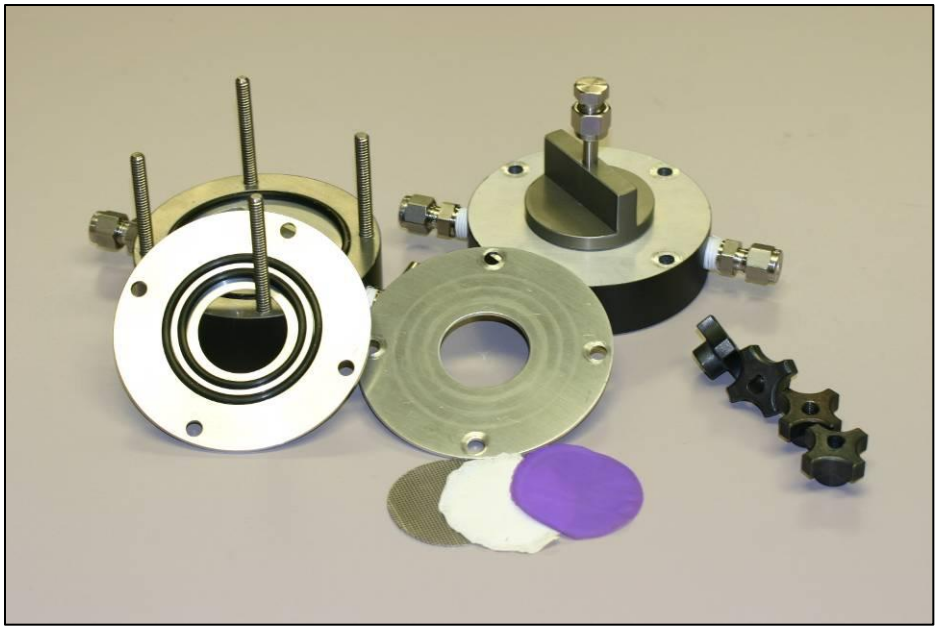

Figure 5. Hybrid test method using DVB sorption pad to collect contact-transfer analyte within an AVLAG cell.

2.2.3 Contact Testing

A diagram of the new contact test cell is shown in Figure 1. The contact test fixture consists of a disposable polycarbonate Petri dish lined with a 2 in. diameter PTFE circle. A previously prepared DVB sorbent pad was placed on the PTFE liner and covered with a 2 in. diameter latex swatch. The swatch was contaminated with a single 10 µL droplet of VX and covered with a 1 in. diameter PTFE circle. The circle was a protective layer for a 1 lb stainless steel weight. The entire device was housed within an inverted 8 oz glass jar. Temperature control was achieved via a VWR incubator that had been modified with sliding shelves to facilitate access to the individual jars. After 4 h of contact, the jar was removed, whereupon the sorbent pad and the PTFE liner were extracted in 20 mL of acetone for 30 min. Analysis was conducted via GC-FPD.

Other contact configurations were examined, including application of 2 psi contact pressure, the addition of an annular ring, and application of no additional pressure. The 2 psi contact pressure experiment was the same as the 1 psi setup, except double the mass weight was applied as the contact pressure. In the annular ring test, a stainless steel washer was used to hold the swatch edges without putting direct pressure on the contamination zone. In the no-pressure test, no additional forces were applied to connect the layers. The annular ring and no-pressure configurations are illustrated in Figure 6.

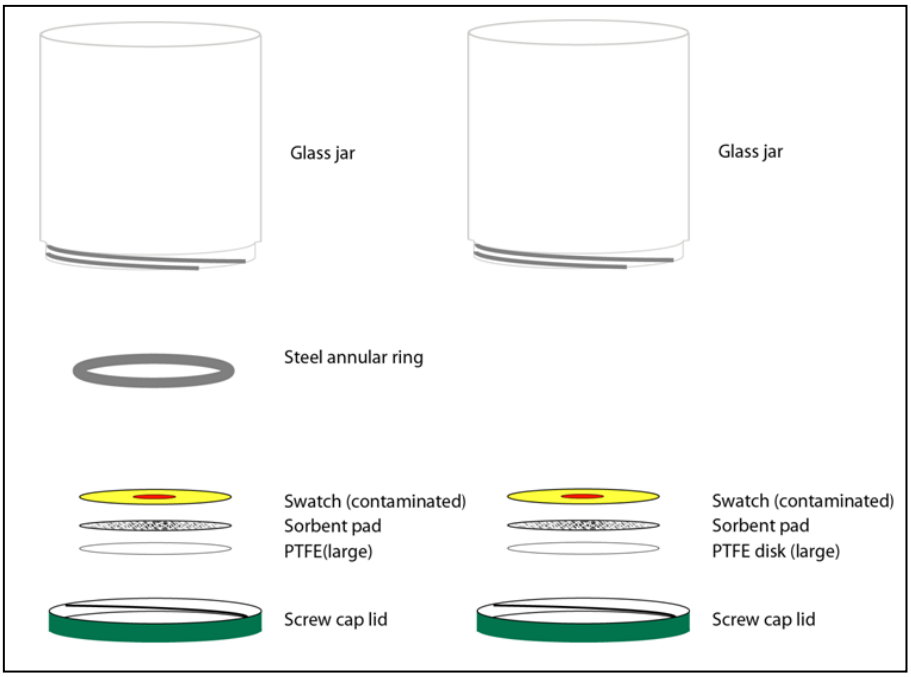

Figure 6. Two additional contact test setups: annular ring (left) and no pressure (right).

2.2.4 Quality Controls

Analytical permeation testing was conducted in accordance with ISO 17025 quality control guidelines. Toxicological testing was conducted under the auspices of the ECBC IACUC and included standards for animal testing. Multiple quality control steps were incorporated into the testing to increase confidence in the data. The quality control processes and results are presented in Section 4.

3. STATISTICAL APPROACH

3.1 Student's *t* Test and Welch's *t* Test

Student's *t* test is a standard statistical approach for comparing two data sets. In this method, it is assumed that the data sets are normally distributed, have equal variances, are independent, and contain the same number of data points. In cases where the variances are not equal, the more-complex Welch's *t* test is appropriate.

Both approaches return a *p* value, which is used to determine if the means of the two data groups are statistically different. The *p* value is the probability of the observed result arising by chance, and it indicates whether there is sufficient evidence to reject the null hypothesis. The null hypothesis states that the mean value is the same for both data sets. A large *p* value indicates that there is insufficient evidence to reject the null hypothesis; that is, the data sets are not statistically different. A *p* value less than the α value (typically 0.05) indicates that it is unlikely that the difference between the data set mean values is the result of the coincidence of random sampling. This is sufficient evidence to reject the null hypothesis and to accept that the data sets have mean values that are statistically different from each other.

3.2 Tukey-Kramer Honestly Significantly Different (HSD) Test

The Tukey-Kramer HSD test provides additional information, beyond the standard *t* tests, to indicate whether data sets are statistically different from one another. The Tukey-Kramer approach is a conservative, single-step method in which each result is compared pairwise to all others. The Tukey-Kramer HSD test can accommodate multiple categories of data, each with different numbers of replicates. This method is used to calculate a critical value to evaluate whether differences between any two pairs of means are significant. The critical value is determined using a range statistic, the mean square error from the overall *F* test, and the sample size for each group. One of the outputs from the Tukey-Kramer analysis is a categorization table of alphabetical letters. Groups that share a letter are statistically similar, and groups that do not share a letter are statistically different.

The software used to perform the analyses in this report was SAS JMP V9.02 (SAS Institute; Cary, NC). This software package provided the tools to perform both versions of the *t* test, the Tukey-Kramer HSD analysis, and graphical output.

3.3 Censored Data and Data Transformations

Permeation testing involves the analysis of a contaminant in a sample extraction. Due to sample, material, and test method variances, some studies could result in a standard deviation (SD) greater than the mean value. Such data sets would indicate that the data distribution could include negative values. However, it is physically impossible to have a negative quantity of contaminant. Therefore, the data may not have a normal distribution, and it may require transformation to meet the requirements for a particular statistical analysis test. Because the data are required to be greater than or equal to zero, it is considered to be left-censored data. Left-censored data are commonly managed using a log-transformation, which removes the issue of negative numbers.[20]

4. QUALITY CHECKS

Multiple steps were incorporated into this program to increase the confidence in the data, including a statistical design of experiments for test planning, purity analysis, verification of deposited mass, logging of environmental conditions, positive- and negative-control samples, sorbent pad uptake and extraction efficiencies, sorption pad water-uptake variance, and analytical quality control.

4.1 Purity Analysis

Vials of VX were obtained from the Chemical Transfer Facility (CTF), and initial purity was documented by CTF personnel. CTF personnel check the purity of each lot annually and issue a certificate of analysis that is valid as long as the vials remain sealed. Once a vial containing agent has been opened, additional purity verification is obtained via ^{31}P-nuclear magnetic resonance (^{31}P-NMR). The VX used during the toxicological evaluations came from previously opened containers; therefore, purity was measured on the day of testing. A fresh vial was opened for each analytical permeation test to avoid the purity issues noted during the toxicological evaluation.

The VX purity analysis is provided in Table 3, including the experiment, the analysis method, date, purity, agent lot, and laboratory where the VX was used.

Table 3. Purity Analysis for VX

Experiment	Method	Date	Purity (%)	Lot	Laboratory
Initial receipt	^{31}P-NMR	22 May 2008 (Memo from CTF)	90.4	VX-U-8042-CTF-N	Toxicology
Direct contact	^{31}P-NMR	8 December 2008	86.0		
Initial receipt	^{31}P-NMR	16 April 2009 (Memo from CTF)	96.2	VX-U-7330-CTF-N	
Offset	^{31}P-NMR	12 November 2009	65.1		
Initial receipt	^{31}P-NMR	24 February 2011 (Memo from CTF)	96.2	VX-U-7330-CTF-N	Analytical permeation
Vapor-only	New vial	7 March 2011			
Hybrid	New vial	8 March 2011			
1 psi and characterization	New vial	10 March 2011			
Contact configurations	New vial	10 May 2011			

4.2 Verification of Deposited Mass

As an additional quality check, the deposition method was characterized and analyzed for the first 3 test days. This was accomplished by contaminating a PTFE disk with a known mass of VX and performing an immediate extraction. The results (Table 4) provided information regarding the accuracy of the deposition and extraction procedures with use of a nonsorbing and nonreacting substrate. The percentage recovered mass was calculated by dividing the sample recovered mass by the theoretical 100% recovery mass for 10 µL of contaminant. The results indicated that the process resulted in an average accuracy, within ~20% of the target, with a variance of less than 10%.

Table 4. Deposition and Extraction Quality Check Results

Test Day	Recovered Mass (mg)	Percentage Recovered Mass Compared to Theoretical (%)
1	10.35	103.5
2	12.06	120.6
3	11.99	119.9
Average	11.47 mg	114.7%
SD	0.97 mg	9.67%
RSD	8.44%	8.44%

RSD, relative standard deviation

4.3 Environmental Condition Logs

The environmental temperature was set to 37.2 °C for all permeation tests. For each experiment, this temperature was logged at least once every 60 s. An example of the environmental control is presented as a histogram in Figure 7. The *x*-axis shows temperature bins with a 0.25 °C resolution; the *y*-axis shows the histogram count of measurements for each temperature bin as a percentage of the whole. Here, the red bars indicate the vapor-only experiment, and the green bars indicate the hybrid experiment. The purple bars indicate the 1 psi contact pressure test-method experiment and the efficiency characterization tests. The blue bars indicate the other contact test analogs, including the 2 psi, the annular ring, and the no-pressure configurations.

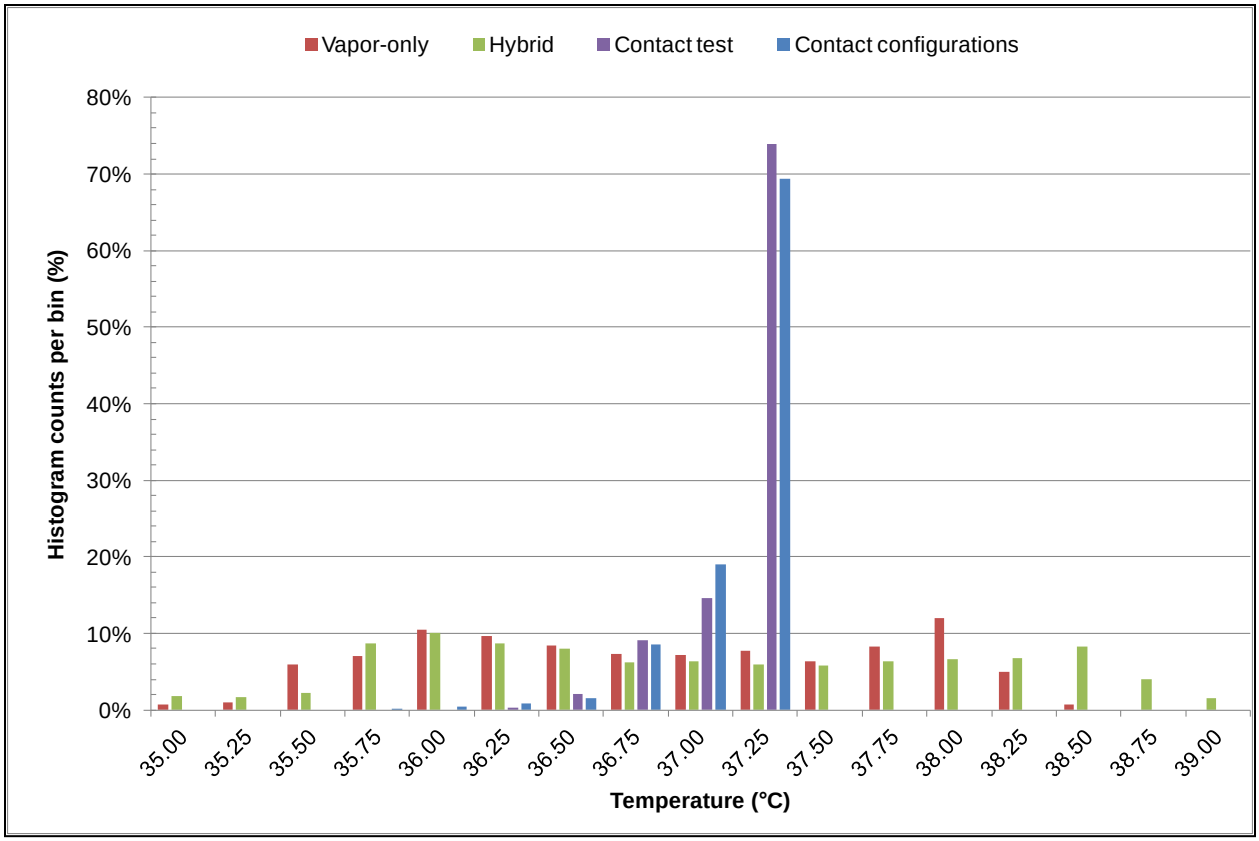

Figure 7. Histogram plot of environmental temperature conditions for experiments.

4.4 Positive-Control Samples

Positive-control animals were included as part of the toxicological testing. For these animals, 1 µL of VX was applied to the rabbit's back, and no protective layer was included. Latex swatches are normally used as a positive-control material for analytical permeation testing. Positive results were achieved with all latex swatches tested; no additional positive-control samples were included for the analytical permeation tests. Results obtained from positive-control animals are detailed in Section 5.2.2.

4.5 Negative-Control Samples

Negative-control samples were included for analytical permeation and toxicological testing. For negative-control samples, the entire test process was completed using an uncontaminated latex swatch to identify any potential positive results from cross-contamination.

4.5.1 Negative-Control Toxicological Animals

A total of three animals were used as negative controls during these studies. None of the negative-control animals displayed any toxicological effects. Results for negative-control animals are detailed in Section 5.2.3.

4.5.2 Negative-Control Analytical Permeation Samples

The negative-control results are summarized in Table 5. The uptake-efficiency negative control seems to have been accidently spiked during testing. The mass measured in the sample was similar to the uptake-efficiency samples that had been characterized that day. None of the other negative-control samples had measureable VX in the sample that was above the quantification limit; these samples were marked as below the quantification limit (BQL).

Table 5. Summary of Negative-Control Results for Analytical Permeation Testing

Negative-Control Test Configuration	Position	PASB	Mass Analyzed (µg)
DVB uptake efficiency	–[a]	1471	11.8
PTFE uptake efficiency	–[a]	1482	BQL
Vapor-only	9	1421	BQL
Hybrid vapor tube	11	1447	BQL
Hybrid DVB	11	1435	BQL
1 psi contact pressure DVB	11	1460	BQL
Contact configuration (2 psi)	11	1638	BQL

PASB, internal sample-tracking number used by ECBC Permeation and Analytical Solutions Branch
[a] Not applicable.

4.6 Extraction and Uptake Efficiencies

Extraction efficiency and uptake-efficiency tests were methods for characterizing the DVB pad performance. These characterizations enabled documentation of the accuracy of results obtained with the DVB sorption pad.

For the extraction efficiancy test, DVB pads were prepared IAW the manufacturer's instruction. Each pad was placed in the bottom of an 8 oz jar. The DVB was spiked with 50 µL of a 0.25 mg/mL solution of VX in acetone. The solvent was allowed to dry before the jar was closed for 4 h. Next, 20 mL of solvent was added, and the candidate latex was extracted for 30 min. An aliquot was then removed for analysis. A negative control and five positive controls were also included. The negative control was a DVB extraction with no analyte spiking. The positive controls were 20 mL of solvent spiked with 50 µL of a 0.25 mg/mL solution without a DVB pad. Screening was performed with 10 replicates in which acetone was the extraction solvent.

Several additional steps were needed for the uptake-efficiency test. PTFE disks of 2 in. diameter were spiked with 50 µL of a 0.25 mg/mL solution of VX in acetone. Once the solvent had evaporated, the disk was covered with the prepared DVB pad. A second PTFE disk was used as a spacer between the pad and the 1 lb weight. The entire apparatus was sealed in a glass jar and incubated at 37.1 °C for 4 h. Once the contact time was complete, the candidate material and spacer were extracted together in 20 mL of acetone. The originally contaminated PTFE disk was extracted in 20 mL of acetone in a separate jar. A negative control and five positive controls were also included. The negative controls were a combined DVB and PTFE extraction with no analyte spiking. The positive controls were PTFE disks that had been spiked with contaminant and extracted immediately in 20 mL of solvent spiked with 50 µL of a 0.25 mg/mL VX solution without a DVB pad. Characterization was performed with 10 replicates.

To calculate extraction efficiency, extracted samples were compared to a known standard. The measured concentration for each extraction efficiency sample and solvent spike was multiplied by the solvent volume to produce the total mass of contaminant recovered. The total masses of the solvent spike samples were averaged to yield the known standard target of analysis in the absence of the sorbent layer. The extracted mass for each extraction efficiency sample was divided by the average of the spike samples to produce a ratio, which was the extraction efficiency percentage for that particular sample. The ratios for all extraction efficiency samples were averaged to obtain the overall extraction efficiency performance for the sorbent.

Uptake-efficiency calculations also required comparison of the extracted sample to a known standard. The measured concentration for each uptake-efficiency sample and PTFE spike was multiplied by the solvent volume to yield the total mass of contaminant recovered. The total masses of the PTFE spike samples were averaged to produce the known standard target of analysis in the absence of the sorbent layer. The extracted mass for each uptake-efficiency sample was divided by the average of the PTFE spike samples to produce a ratio, which was the uptake-efficiency percentage for that particular sample. The ratios for all uptake-efficiency samples were averaged to obtain the overall uptake-efficiency performance for the sorbent.

The extraction and uptake-efficiency results are summarized in Table 6. The individual sample results, including analyzed mass and efficiency results for extraction uptake, are shown in Table 7. The uptake-efficiency negative control appears to have been accidently spiked at the same level as the other uptake samples.

Table 6. Summary of Extraction and Uptake-Efficiency Characterization Results for DVB Pads

Extraction Efficiency			**Uptake Efficiency**		
n	**Average Recovery (%)**	**Range (%)**	***n***	**Average Recovery (%)**	**Range (%)**
10	82.5	77.5–91.4	10	78.9	62.0–84.0

Table 7. Individual Extraction and Uptake-Efficiency Characterization Results

Characterization Type	PASB	Mass Analyzed (µg)	Efficiency (%)
DVB uptake	1472	12.0	82.6
	1473	12.0	82.6
	1474	12.0	82.6
	1475	11.6	79.9
	1476	12.2	84.0
	1477	12.0	82.6
	1478	11.8	81.3
	1479	9.0	62.0
	1480	11.2	77.1
	1481	10.8	74.4
Uptake negative	1471	11.8[a]	–[b]
PFTE uptake	1483	BQL	–[b]
	1484	BQL	–[b]
	1485	BQL	–[b]
	1486	BQL	–[b]
	1487	BQL	–[b]
	1488	BQL	–[b]
	1489	BQL	–[b]
	1490	BQL	–[b]
	1491	BQL	–[b]
	1492	BQL	–[b]
PTFE uptake negative	1482	BQL	–[b]
PTFE immediate extract	1498	14.4	–[b]
	1499	15.6	–[b]
	1500	13.8	–[b]
	1501	15.2	–[b]
	1502	13.6	–[b]
Solvent spike	1493	15.0	–[b]
	1494	16.2	–[b]
	1495	16.0	–[b]
	1496	16.0	–[b]
	1497	15.6	–[b]
DVB extraction efficiency	1503	12.6	79.9
	1504	14.4	91.4
	1505	13.0	82.5
	1506	12.8	81.2
	1507	13.2	83.8
	1508	13.2	83.8
	1509	12.2	77.4
	1510	13.0	82.5
	1511	13.0	82.5
	1512	12.6	79.9

PASB, internal sample tracking number used by ECBC Permeation and Analytical Solutions Branch
[a] Negative control appears to have been accidently spiked.
[b] Not applicable.

4.7 Water-Uptake Variance

The preparation method for the DVB pads involved filtering a series of solvents through the pad; the last solvent was water. The manufacturer cautions against filtering the water step to the point of dryness because this would affect the material performance. Therefore, the pads remained moist after the final filtering step. The amount of water uptake was measured in a series of pads to document the variance in uptake. This was achieved by weighing each pad dry, completing the preparation steps, and reweighing the moistened pads. All weighing steps were conducted using a calibrated analytical balance. Individual weights for the 20 pads evaluated are listed in Table 8. Results from this evaluation indicated that the variance in water uptake was less than 8%.

Table 8. Individual Pad Mass Before and After Preparation Steps To Document Water-Uptake Variance

Pad Number	Dry Mass (g)	Wet Mass (g)	Water-Uptake Mass (g)
1	0.29980	0.91510	0.61530
2	0.29950	0.88600	0.58650
3	0.30167	0.98300	0.68133
4	0.29860	0.94200	0.64340
5	0.28210	0.76447	0.48237
6	0.29893	0.97860	0.67967
7	0.26980	0.83876	0.56896
8	0.29631	0.90983	0.61352
9	0.29475	0.92270	0.62795
10	0.29053	0.91151	0.62098
11	0.28597	0.88251	0.59654
12	0.29762	0.88424	0.58662
13	0.27789	0.82972	0.55183
14	0.29340	0.91323	0.61983
15	0.29363	0.92931	0.63568
16	0.29110	0.87780	0.58670
17	0.29122	0.91947	0.62825
18	0.28163	0.86270	0.58107
19	0.28743	0.88062	0.59319
20	0.27107	0.85886	0.58779
Average			0.60437 g
SD			0.044004 g
RSD			7.3%

RSD, relative standard deviation

4.8 Analytical Procedures

Periodically throughout the sample analysis process, a continuing calibration verification (CCV) standard was analyzed to ensure the continued validity of the calibration curve (e.g., after every tenth sample). The CCV reference materials were prepared from stock solutions different from the calibration standards. Established method acceptance limits require that the results of the CCV standard analysis must be between 66 and 149% of the theoretically calculated concentration. Test samples analyzed between acceptable CCV standards were considered valid. Test samples analyzed near the failed CCV standards were reanalyzed.

A statistical analysis was performed for the CCV and calibration response data. The results for the CCV and the calibration standards were analyzed, and upper and lower warning limits were established based on 2 SDs from the mean. Upper and lower control limits were also calculated and were established for 3 SDs from the mean.

All CCV and calibration response data included in the report was found to be within established acceptance limits for this method. The summary results for the CCV are plotted in Figure 8 and the summary results for the calibration curve are plotted in Figure 9.

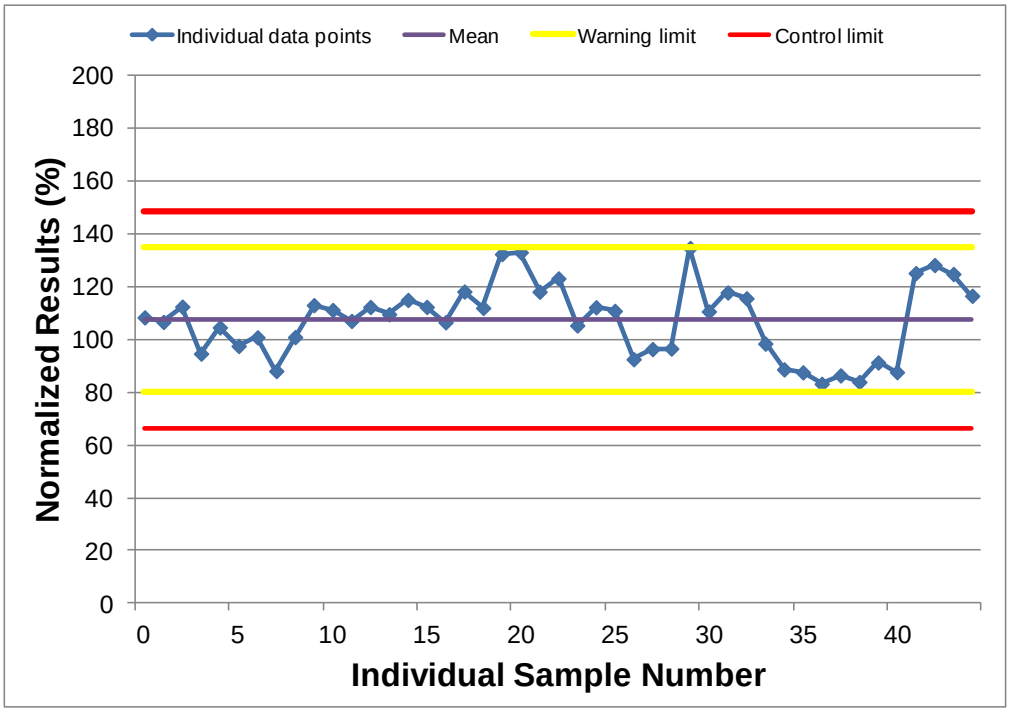

Figure 8. Summary plot for CCV quality data.

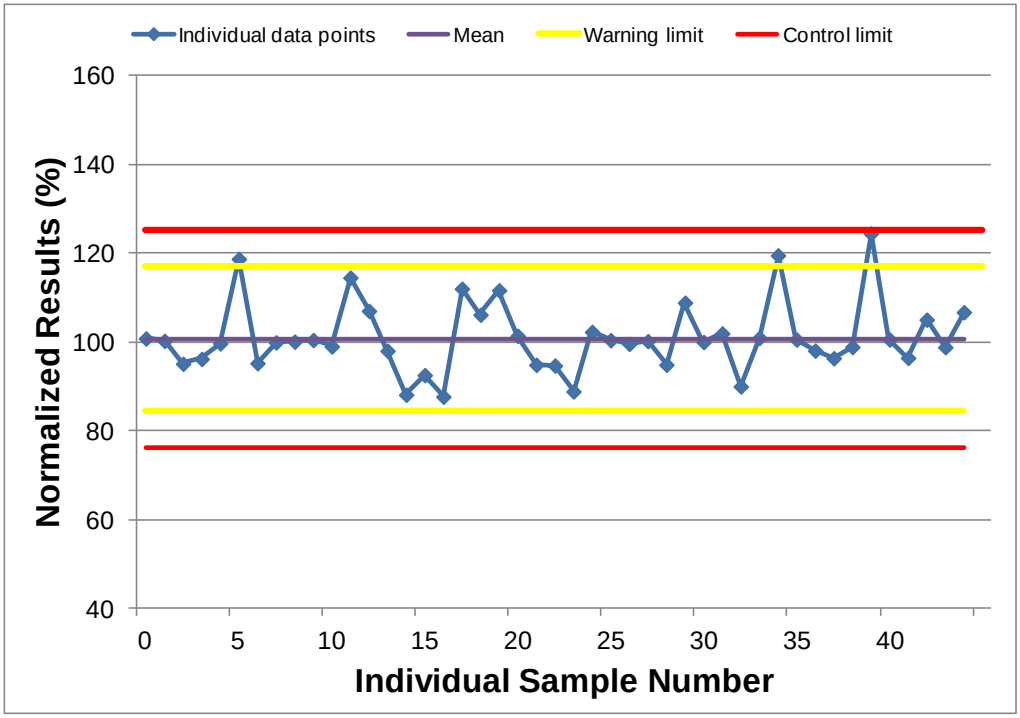

Figure 9. Summary plot for calibration curve quality data.

5. RESULTS

5.1 Toxicological Percutaneous Testing

Acute percutaneous toxicity is defined as the adverse effects that occur within a short time after the dermal application of either single or multiple doses of a substance typically given within a short time period. The test provides information on health hazards likely to arise from short-term exposure by the percutaneous route. For this application, a percutaneous evaluation was used to determine the toxicological effects of VX permeating latex in various configurations. These hazard determinations were accomplished by placing a latex swatch either directly against the test animals' skin or offset from the skin by 1 cm and observing the clinical signs and blood AChE levels during a 4 h exposure. Post-exposure analytical assays were performed on blood plasma and skin tissue. Positive- and negative-control animals were used each test day for data comparisons.

5.2 Test Results

5.2.1 General Toxicological Results

Sections 5.2.2–5.2.5 provide the toxicological results for each test configuration. The first table in each section is a summary of results, and the second table lists the results from individual animals. The tables include the rabbit number, rabbit mass, panel identification number (ID), test date, most-severe toxic sign noted, and sign onset time. The post-exposure assay section provides results for the plasma and skin assays. For animals with quantifiable contaminant in their plasma, the total contaminant in the rabbit plasma was calculated (in nanograms). This value was calculated by multiplying the plasma assay results (nanograms of contaminant per gram of plasma) by the estimated total mass of plasma (grams) in a rabbit, as shown in eq 1. Plasma mass per rabbit was dependent on the plasma volume per rabbit mass and the plasma density:

$$\frac{\text{contaminant mass}}{\text{plasma mass}} \times \frac{\text{plasma volume}}{\text{rabbit mass}} \times \frac{\text{plasma mass}}{\text{plasma volume}} \times \text{rabbit mass} = \text{contaminant mass} \quad (1)$$

The values used for the calculation of contaminant mass were 38.8 mL of plasma per kilogram of rabbit body mass and a plasma density of 1.025 g/mL.[21] The total mass of contaminant accounted for was calculated by summing the results from the swab assay, plasma assay (per rabbit), skin assay, and panel extraction.

The periodic AChE assay results and the subsequent depression severity ratings are also provided for each rabbit in Sections 5.2.2–5.2.5. These are color-coded to illustrate the trends, ranging from yellow (mild), to orange (moderate), to red (severe).

For rabbits in the direct-contact and positive-control test groups, an additional table is provided along with the onset time for toxic sign categories. The categories and associated individual toxic signs are noted in Table 1. For most animals, localized fasciculations were not noted. It was difficult to identify these ambiguous skin twitches because the swatch occluded the exposure site. Therefore, in most cases, fasciculations could only be observed when the panel was removed at 4 h. Further compounding the complexity of this observation, the process of removing a panel can also induce localized fasciculations. For the direct-contact and positive-control animals, any localized fasciculations were overtaken by more-severe signs.

The results for the rabbit percutaneous tests are summarized in Table 9. As expected, the rabbits in direct contact with the exposed latex progressed through a series of toxic signs for nerve-agent exposure that culminated in lethality. Severe AChE depression is defined here as having less than 25% active enzyme remaining. The agent in blood results are presented as a concentration of mass contaminant per mass of blood. The agent in skin results are presented as the total mass of contaminant measured.

Table 9. Summary of Results for Percutaneous Rabbit Tests

Analysis	Direct Contact[a]	Offset[a]
Toxic signs	8/8 Lethality	8/10 None 2/10 Ambiguous mild signs
AChE depression	8/8 Severe (within 2 h)	10/10 None (within 2 h) 2/10 Severe (within 4 h)
Geometric mean agent in blood plasma	8/8 1.023 ng/g	2/10 0.649 ng/g
Geometric mean agent in skin under test swatch	8/8 58,995 ng	10/10 2,615 ng

[a] Results include number of rabbits affected/total rabbits tested.

5.2.2 Positive-Control Animals

Three rabbits were tested with neat VX applied directly to the skin on their backs, with no barrier present, and all three animals died during the course of testing. Contaminant was measured in the plasma samples, and microgram quantities of contaminant were measured in the skin for all three animals. Severe AChE depression was measured in all three rabbits. None of the nine AChE blood samples were considered outliers. The positive-control panel results are summarized in Table 10, and results for individual rabbits are presented in Table 11. The results for elapsed time to toxic sign categories are presented in Table 12.

Table 10. Summary Results for Positive-Control Evaluations of Rabbits

Sign	*n*	Geometric Mean Onset Time (min)	Range (min)
Death	3/3	43	31–56
Post-Exposure Assays	***n***	**Geometric Mean**	**Range**
Plasma assay (ng/g)	3/3	1.4565	1.1072–1.9494
Skin assay (ng)	3/3	38,047	29,318–59,082
AChE Activity			
Time (min)	**Mean (%)**	**SD (%)**	**Range (%)**
0	100.0	–	–
10	29.3	29.8	7.6–64.3
20	11.9	22.0	5.2–44.3
30	6.2	1.6	5.1–8.2
AChE Depression Severity	Severe 3/3		

Table 11. Toxicological Results for Individual Positive-Control Rabbits

	Rabbit Number		
	77	**78**	**220**
Mass (kg)	2.78	2.67	2.54
Date	1 Jul 2008	1 Jul 2008	11 Nov 2009
Material	Positive	Positive	Positive
Toxic signs	Death	Death	Death
Time to death (min)	31	56	45
Post-Exposure Assays			
Plasma assay (ng/g)	1.9494	1.1072	1.4315
Plasma assay per rabbit (ng)	215.5	117.6	144.6
Skin assay (ng)	29,318	31,797	59,082
AChE Activity (%)			
10 min	7.6	64.3	51.6
20 min	5.2	44.3	7.4
30 min	5.1	8.2	5.7
AChE Depression Severity Category	3	3	3

Table 12. Elapsed Time to Toxic Effects for Individual Positive-Control Rabbits

Rabbit Number	Elapsed Time to Effects (min)					
	Local Fasciculations	**Discrete CNS**	**Diffuse CNS**	**Cardio-respiratory**	**Moribund**	**Death**
77	6	24	20.5	27	26	31
78	7	41	37.5	46	38	56
220	25	31	36	43	31	45
Count	3/3	3/3	3/3	3/3	3/3	3/3
Geometric Mean (min)	10	31	30	38	31	43
Range (min)	6–25	24–41	21–38	27–46	26–38	31–56

CNS, central nervous system

5.2.3 Negative-Control Animals

Three rabbits completed the entire test process with various panels that had not been contaminated. None of the animals displayed any toxic signs. For all of the plasma and skin samples analyzed, contaminant was not measured above the 0.5 ng/g quantification limit. No decrease in AChE activity was measured for any of the animals. None of the 19 AChE blood samples were considered outliers. The negative-control panel results are summarized in Table 13, and the results for individual rabbits are presented in Table 14.

Table 13. Rabbit Summary Results for Negative-Control Evaluations

Sign	***n***	**Geometric Mean Onset time (min)**	**Range (min)**
No signs	3/3	–[a]	–[a]
Post-Exposure Assays	***n***	**Geometric Mean**	**Range**
Plasma assay (ng/g)	2/2	BQL	–[a]
Skin assay (ng)	1/1	BQL	–[a]
AChE Activity			
Time (min)	**Geometric Mean (%)**	**SD (%)**	**Range (%)**
0	100.0	–[a]	–[a]
30	100.4	7.3	95.8–108.9
60	96.0	4.4	93.3–101.1
90	95.1	–[a]	–[a]
120	98.7	4.7	93.5–102.6
240	97.1	3.2	94.1–100.4
360	111.7	5.3	108–115.5
1440	107.2	4.8	103.9–110.7
2880	111.9	4.7	108.6–115.2
AChE Depression Severity	None 3/3		

[a] Not applicable.

Table 14. Toxicological Results for Individual Negative-Control Rabbits

	Rabbit Number		
	75	**76**	**208**
Mass (kg)	2.68	2.61	2.31
Date	1 Jul 08	1 Jul 08	10 Nov 09
Material	Latex sheet	Latex sheet	Latex sheet
Panel ID	08	05	08
Method	Direct contact	Direct contact	Offset
Toxic signs	None	None	None
Onset time	–[a]	–[a]	–[a]
Post-Exposure Assays			
Plasma assay (ng/g)	BQL	–[b]	BQL
Skin assay (ng)	–[b]	–[b]	BQL
AChE Activity (%)			
30 min	95.8	96.9	108.9
60 min	93.8	933	101.1
90 min	–[b]	–[b]	95.1
120 min	93.5	100.3	102.6
240 min	96.9	94.1	100.4
360 min	115.5	108.0	–[c]
1440 min	110.7	103.9	–[c]
2880 min	108.6	115.2	–[c]
AChE Depression Severity Category	0	0	0

[a] Not applicable.
[b] Not analyzed/samples not collected.
[c] Not applicable; animal was euthanized at 240 min.

5.2.4 Direct-Contact Results

Eight rabbits were tested with latex swatches directly against their skin. All eight rabbits died and were found to have microgram levels of VX in the skin. Agent was measured in plasma samples from all of the animals to be above the 0.5 ng/g quantification limit. In addition, severe depression of AChE activity was measured in all eight rabbits within this test group, and none of the 39 AChE blood samples were considered to be statistical outliers. The direct-contact results are summarized in Table 15, and the results for individual rabbits within this test group are presented in Table 16. The elapsed times to the occurrence of toxic effects are shown in Table 17.

Table 15. Summary Results for Latex in Direct Contact with Rabbits

Toxic Sign	***n***	**Geometric Mean Onset time (min)**	**Range (min)**
Death	8/8	159	87–193
Post-Exposure Assays	***n***	**Geometric Mean**	**Range**
Plasma assay (ng/g)	8/8	1.023	0.6638–1.6928
Skin assay (ng)	8/8	58,995	36,804–85,099
AChE Activity			
Time (min)	**Geometric Mean (%)**	**SD (%)**	**Range (%)**
0	100.0	–[a]	–[a]
30	97.5	8.6	87.0–114.4
60	99.1	10.0	85.0–115.2
90	96.3	11.6	79.8–114.0
120	99.5	11.9	79.6–120.5
240	70.5	41.5	7.5–129.5
AChE Depression Severity	None 8/10 Severe 2/10		

[a] Not applicable.

Table 16. Toxicological Results for Rabbits in Direct Contact with Latex Swatches, 1 July 2008

	Rabbit Number							
	65	**66**	**68**	**70**	**71**	**72**	**73**	**74**
Mass (kg)	2.75	2.66	2.61	2.72	2.78	2.82	2.55	2.65
Material	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet
Panel ID	4	2	9	10	6	7	3	1
Method	Direct contact	Direct contact	Direct contact	Direct contact	Direct contact	Direct contact	Direct contact	Direct contact
Toxic signs	Death	Death	Death	Death	Death	Death	Death	Death
Time to death (min)	166	171	193	163	168	87	172	184
Post-Exposure Assays								
Plasma assay (ng/g)	0.6638	0.7730	1.3239	1.4517	1.6928	0.8672	0.9745	0.8509
Plasma assay per rabbit (ng)	72.6	81.8	137.4	157.0	187.2	97.3	98.8	89.7
Skin assay (ng)	36,804	43,087	77,026	79,170	61,019	85,099	51,678	56,542
AChE Activity (%)								
10 min	82.4	97.7	99.5	106.8	106.3	95.5	97.2	96.7
20 min	99.9	86.4	86.9	106.0	102.6	82.5	95.4	94.1
30 min	90.9	97.9	85.8	100.0	98.9	48.6	92.7	88.6
60 min	87.4	68.2	82.8	87.9	69.5	8.0	75.3	62.4
120 min	7.5	6.8	8.9	6.0	7.5	–[a]	7.7	8.5
AChE Depression Severity Category	3	3	3	3	3	3	3	3

[a] Not applicable.

Table 17. Elapsed Time to Toxic Effects for Direct-Contact Rabbits

Rabbit No.	Elapsed Time to Effects (min)					
	Local Fasciculations	Discrete CNS	Diffuse CNS	Cardio/ Respiratory	Moribund	Death
65	113	140	130	–[a]	145	166
66	75	128	127.5	136	129	171
68	101	155	141	165	163	193
70	101	133	110	157	1390	163
71	97	135	111.5	151	138	168
72	71	84	73.5	81	77	87
73	67	161	107	170	159	172
74	108	139	135	174	170	184
Count	8/8	8/8	8/8	7/8	7/8	8/8
Geometric mean (min)	90	132	115	144	182	159
Range (min)	67–113	84–161	74–141	81–174	77–1390	87–193

[a] Not observed.

5.2.5 Offset Results

Ten rabbits were tested with latex swatches offset from their skin by 1 cm. Two of the animals displayed localized fasciculations that were identified after the swatch was removed from the skin. Contaminant was measured to be above the 0.5 ng/g quantification limit in the plasma samples from the two animals that displayed fasciculations. Contaminant was measured in the skin of all animals. Severe depression of AChE activity was measured in two of the animals. Of the 50 total AChE blood samples, none were considered to be statistical outliers. The offset results are summarized in Table 18, and the results for individual rabbits are presented in Table 19.

Table 18. Summary Results for Latex Offset from the Skin of Rabbits

Toxic Sign	*n*	Geometric Mean Onset time (min)	Range (min)
No signs	8/10	–[a]	–[a]
Fasciculations	2/10	247	246–248
Post-Exposure Assays	***n***	**Geometric Mean**	**Range**
Plasma assay (ng/g)	2/10	0.6489	0.3539–1.1897
Skin assay (ng)	10/10	2,615	456–14,184
AChE Activity (%)			
Time (min)	**Geometric Mean (%)**	**SD (%)**	**Range (%)**
0	100.0	–[a]	–[a]
30	97.5	8.6	87.0–114.4
60	99.1	10.0	85.0–115.2
90	96.3	11.6	79.8–114.0
120	99.5	11.9	79.6–120.5
240	70.5	41.5	7.5–129.5
AChE Depression Severity	None 8/10 Severe 2/10		

[a] Not applicable.

Table 19. Toxicological Results for Rabbits with Offset Latex Sheet Swatches, 10 November 2009

	Rabbit No.									
	227	**229**	**230**	**238**	**239**	**240**	**241**	**242**	**243**	**244**
Mass (kg)	2.55	2.57	2.51	2.81	2.93	2.94	2.96	2.91	2.58	2.45
Material	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet	Latex sheet
Panel ID	02	10	01	04	09	07	05	03	06	11
Method	Offset	Offset	Offset	Offset	Offset	Offset	Offset	Offset	Offset	Offset
Toxic signs	None	None	None	None	None	Fasc	None	None	None	Fasc
Onset time (min)	–[a]	–[a]	–[a]	–[a]	–[a]	246	–[a]	–[a]	–[a]	248
Post-Exposure Assays										
Plasma assay (ng/g)	BQL	BQL	BQL	BQL	BQL	1.1897	BQL	BQL	BQL	0.3539
Plasma assay per rabbit (ng)	BQL	BQL	BQL	BQL	BQL	139.1	BQL	BQL	BQL	34.5
Skin assay (ng)	2,515	3,131	1,386	807	9,050	14,184	456	2,596	2,896	3,857
AChE Activity (%)										
30 min	94.9	95.6	93.5	94.8	87.0	108.7	97.3	103.9	114.4	88.7
60 min	96.9	86.9	109.2	95.3	99.6	95.5	115.2	100.4	111.9	85.0
90 min	96.9	85.4	109.3	98.5	95.7	93.7	110.8	85.5	114.0	79.8
120 min	97.7	84.5	101.0	120.5	103.9	98.1	99.4	112.9	103.8	79.6
240 min	101.9	90.9	113.3	129.5	106.3	7.5	106.1	116.7	110.2	20.5
AChE Depression Severity Category	0	0	0	0	0	3	0	0	0	3

Fasc, fasciculations
[a] Not applicable.

5.3 Analytical Permeation Results

5.3.1 Summary Results

A summary of the results comparing the vapor-only, hybrid, and contact test methods is presented in Table 20. This table includes the configurations, numbers of replicates, geometric means of the measured permeated mass, SDs, RSDs, and percentages measured compared to the initial contamination of 10,000 µg.

A summary of the results comparing the various contact test configurations of 1 psi, 2 psi, annular ring, and no-pressure is presented in Table 21. This table includes the configurations, numbers of replicates, geometric means of the measured permeated mass, SDs, RSDs, and percentages measured compared to the initial contamination of 10,000 µg. The 1 psi test results are included in both tables to facilitate comparisons.

Sections 5.3.2–5.3.5 provide the individual analytical permeation results for each test configuration. Each section represents an independent test day and includes a table with the configuration, sample position number, internal tracking number used by the ECBC PASB, and mass analyzed for the sample.

Table 20. Summary Results Comparing Vapor, Hybrid, and Contact Test Methods

Configuration	*n*	Geometric Mean Permeated Mass (µg)	SD (µg)	RSD (%)	Percentage of Initial Contamination (%)
Vapor-only	11	51.70	12.8	24.8	0.52
Hybrid	11	1402.3	937.0	66.8	14.02
1 psi contact test	5	8303.7	512.5	6.2	83.04

Table 21. Summary Results Comparing Contact Test Methods with Various Pressure Configurations

Configuration	*n*	Geometric Mean Permeated Mass (µg)	SD (µg)	RSD (%)	Percentage of Initial Contamination (%)
1 psi contact test	5	8303.7	512.5	6.2	83.04
2 psi contact test	5	8212.9	524.1	6.4	82.13
Annular ring	5	1296.6	1246.2	96.1	12.97
No pressure	5	1199.8	1944.8	162.1	12.00

5.3.2 Vapor-Only Results

Eleven latex swatches were evaluated in a vapor-only configuration. The 12th sample, in position 9, was used as a negative control. VX was measured as permeating through all of the contamination samples. In the negative-control sample, VX was measured as BQL. Individual results for the vapor-only test configuration are shown in Table 22. For each sample, the position number, PASB internal sample identification number, and mass analyzed are tabulated. The PTFE sample was prepared during testing as part of the quality control for the deposition tool, but it was not inserted into the permeation rack system; therefore, it did not have a position number.

Table 22. Individual Results for Vapor-Only Test Configuration

Test Configuration	Position	PASB	Mass Analyzed (µg)
Vapor only	1	1422	68.2
	2	1423	48.0
	3	1424	45.5
	4	1425	51.3
	5	1426	64.9
	6	1427	70.7
	7	1428	67.3
	8	1429	53.5
	10	1421	41.4
	11	1430	36.1
	12	1431	37.4
Vapor-only negative control	9	1421	BQL
PTFE spike	–[a]	1434	10,350

[a] Not applicable.

5.3.3 Hybrid Results

For each sample, the position numbers, PASB internal sample identification numbers, and mass analyzed were tabulated. Two analytical samples, a vapor tube and a DVB extraction, were obtained from each latex swatch. For example, the vapor tube and DVB listed for position 3 were obtained from the same latex sample. The results for both sample types are provided in Table 23. The PTFE sample was prepared during testing as part of the quality control for the deposition tool, but it was not inserted into the permeation rack system; therefore, it did not have a position number.

Table 23. Individual Results for Hybrid Test Configuration

Sample Source	Position	PASB	Mass Analyzed (µg)
Hybrid vapor tube	1	1448	1.1
	2	1449	0.9
	3	1450	BQL
	4	1451	BQL
	5	1452	BQL
	6	1453	0.13
	7	1454	BQL
	8	1455	0.6
	9	1456	0.98
	10	1457	0.36
	12	1448	0.12
Hybrid vapor tube negative control	11	1447	BQL
Hybrid DVB	1	1436	1,082
	2	1437	1,505
	3	1438	2,762
	4	1439	998
	5	1440	3,866
	6	1441	1,226
	7	1442	1,363
	8	1443	818
	9	1444	1,721
	10	1445	1,440
	12	1446	699
Hybrid DVB negative control	11	1435	BQL
PTFE spike	–[a]	1459	12,060

[a] Not applicable.

5.3.4 New Contact Test Method Results

For each sample, the position numbers, PASB internal sample identification numbers, and mass analyzed were tabulated. The results for all samples are provided in Table 24. The PTFE sample was prepared during testing as part of the quality control for the deposition tool, but it was not inserted into the permeation rack system; therefore, it did not have a position number.

Table 24. Individual Results for the Newly Developed Contact Test Method

Sample Source	Position	PASB	Mass Analyzed (µg)
1 psi contact pressure DVB	6	1466	8,087
	7	1467	8,650
	8	1468	9,028
	9	1469	7,754
	10	1470	8,061
1 psi contact pressure DVB negative control	11	1460	BQL
PTFE spike	–[a]	1532	11,988

[a] Not applicable.

5.3.5 Comparison of Contact Test Configurations

For each sample, the position numbers, PASB internal sample identification numbers, and mass analyzed were tabulated. The results for all samples are provided in Table 25.

Table 25. Individual Results for Various Contact Test Configurations

Test Configuration	Position	PASB	Mass Analyzed (µg)
No pressure	1	1623	1054
	2	1624	973
	3	1625	776
	4	1626	5182
	5	1627	603
2 psi contact pressure	6	1628	8548
	7	1629	8207
	8	1630	8912
	9	1631	7868
	10	1632	7597
Annular ring	28	1633	831
	29	1634	1426
	30	1635	634
	31	1636	1305
	32	1637	3738
Negative control (2 psi)	11	1638	BQL

6. DISCUSSION

6.1 Toxicological Percutaneous Rabbit

The toxicological results in this report are illustrative to demonstrate the differences between direct-contact and offset testing. Significant differences in toxicological responses were noted between the direct-contact and offset-exposure animals. The purpose of including them in this report is to demonstrate the toxicological effects that could result from direct contact, as opposed to an offset configuration, where vapor would be the predominant transfer vehicle.

It is important to note that the toxicological results were compiled from independent efforts. Each toxicological test was designed for a purpose outside the scope of this report, and methodologies differed between the two tests. These results serve as illustrative examples of the toxicological effects.

However, quantitative comparisons between the direct-contact and offset-configurations are not appropriate. A specific reason to preclude quantitative comparisons of the toxicological data is based on differences in the VX purity. However, due to the large VX doses applied in these experiments, purity is not expected to affect the overall demonstrated toxicity. The percutaneous dosage that is lethal to 50% of test subjects (LD_{50}) of VX on rabbits is 23.3 µg/kg.[22] The average rabbit mass from these studies was 2.68 kg; therefore, the average lethal dose would be 62.5 µg per rabbit. However, each test animal was dosed with 8000 µg of VX. Even accounting for the reduced purity of 65%, the applied VX mass was greater than the lethal mass by a factor of 83.

A second specific reason to preclude quantitative comparison is that the offset experiment was stopped at 4 h. Two of the animals displayed an initial toxic sign of localized fasciculations along with severe AChE depression. It is possible that the toxic signs would have progressed to more significant effects, had the rabbits not been euthanized at 4 h. Miosis was observed in rabbit 240. However, this sign was noted at the same time as the euthanization. It is unclear whether the miosis was caused by the VX, the euthanization solution, or the combination of the two. Therefore, miosis was not included as a VX toxic sign, but it is listed here for completeness.

Although differences in agent purity and experimental timing preclude quantitative comparisons between the direct-contact and offset-exposure configurations, the toxicological results are illustrative of agent permeation and demonstrate the need for contact testing. It should be noted that the toxicological setup is more in line with the hybrid testing configuration given that the swatch was held around the perimeter, and direct pressure was not applied to the contaminated region.

6.2 Analytical Permeation

The analytical permeation studies were designed to be quantitatively compared via statistical methods. The differences in approach encountered in the toxicological evaluations were accounted for in the analytical permeation studies. The same 10 mil rolled latex substrate material and fresh vials of VX were used for each experiment.

Quantitative comparisons were performed using Tukey-Kramer analysis. The location of the circle in a Tukey-Kramer plot indicates the mean of the data group. The size of the circle scales inversely with the standard error and is affected by the number of replicates: the greater the number of replicates, the smaller the circle. Statistical similarity was determined by a *p* value generated by the statistical test and is indicated by overlap of comparison circles. If the intersection angle of two comparison circles was greater than 90°, the data were statistically similar.

The green diamonds within the Tukey-Kramer plots show the mean and the 95% confidence range of the data. The width of the diamond indicates the relative sample size. The median and quartiles are demarcated by the red box plot.

In the first analysis, the contact, hybrid, and vapor-only data sets were compared, as shown in Figure 10. The data were log-transformed as part of the Tukey-Kramer analyses to satisfy the requirements for left-censored data and normal distribution. Evaluation of the plot and the group-level output indicated that the three groups were statistically different. The results were supported by the large differences in mean results for the three test methods. Therefore, the group-level output indicated three groups, with no overlap.

In the second analysis, the 1 psi, 2 psi, annular ring, hybrid, and no-pressure configurations for contact testing were compared, as shown in Figure 11. The data were log-transformed as part of the Tukey-Kramer analyses to satisfy the requirements for left-censored data and normal distribution. The plot and the group-level output indicated two statistically different groups. The results were supported by the large differences in mean results for the various test methods. Therefore, the group-level output indicated two groups, and overlap existed between the direct-contact pressure methods and between the methods that did not include direct pressure.

One potential reason for these pairings is the presence or absence of direct pressure on the contamination area. In the hybrid and annular ring methods, an apparatus holds the swatch and DVB pad together at the edges, but not in the middle. VX has been shown to affect latex, causing it to pucker and pull away from the pad as shown in Figure 12. The contaminated region was not in good contact with the DVB pad, which led to a lower level of measured contaminant and a greater amount of variance. Direct contact pressure on the contaminated area prevents this puckering from occurring and ensures good contact between the swatch and the DVB sorption pad.

The two direct-pressure configurations were likewise paired as statistically similar. It should be noted that the pressures used in this study did not appear to force contaminant through the material, because there was no difference in measured contaminant between the 1 psi and 2 psi configurations.

Permeation of a small molecule through a polymer has been described using diffusion models. Many studies have modeled the behavior as Fickean and dependent on the molecular size of the contaminant, structural substrate characteristics, temperature, substrate path length and thickness, and the concentration gradient.[23, 24]

Fickean diffusion models may not be appropriate for cases in which the substrate swells due to analyte imbibition.[23] During instances of substrate swelling, a combination of Fickean and non-Fickean diffusion rates were observed.[25] However, the factors affecting diffusion did not appear to change.

Similarly, a mixture of Arrhenius equations with dynamic adsorption analysis was used to describe the permeation of the chemical warfare agent sulfur mustard (HD) through glove materials.[26] This approach is dependent on activation energy, temperature, and pre-exponential factors, along with the physicochemical nature of the permeant and its interaction with the substrate medium.

An overview of diffusion models indicates three general cases: Fickean, non-Fickean, and anomalous. The differences in the cases are dependent on the speed of molecular diffusion compared with the changes in the physical nature of the medium.[25]

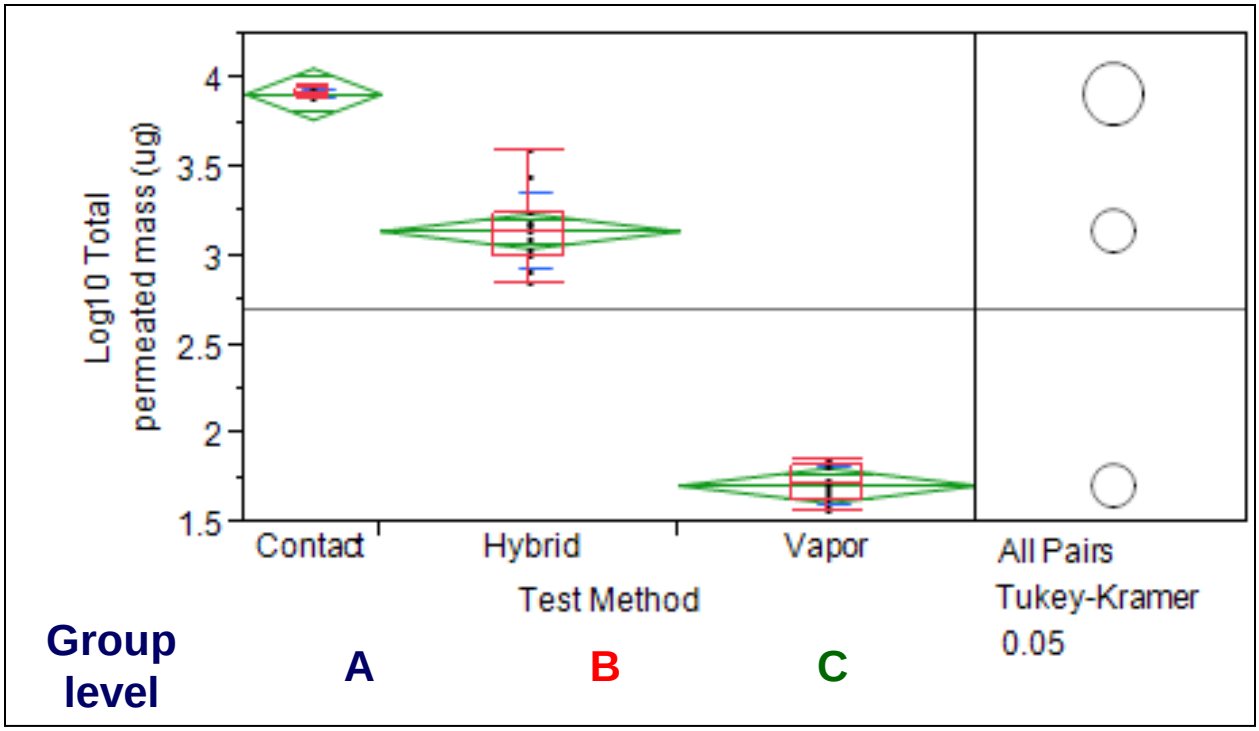

Figure 10. Tukey-Kramer analysis for contact, hybrid, and vapor-only data.

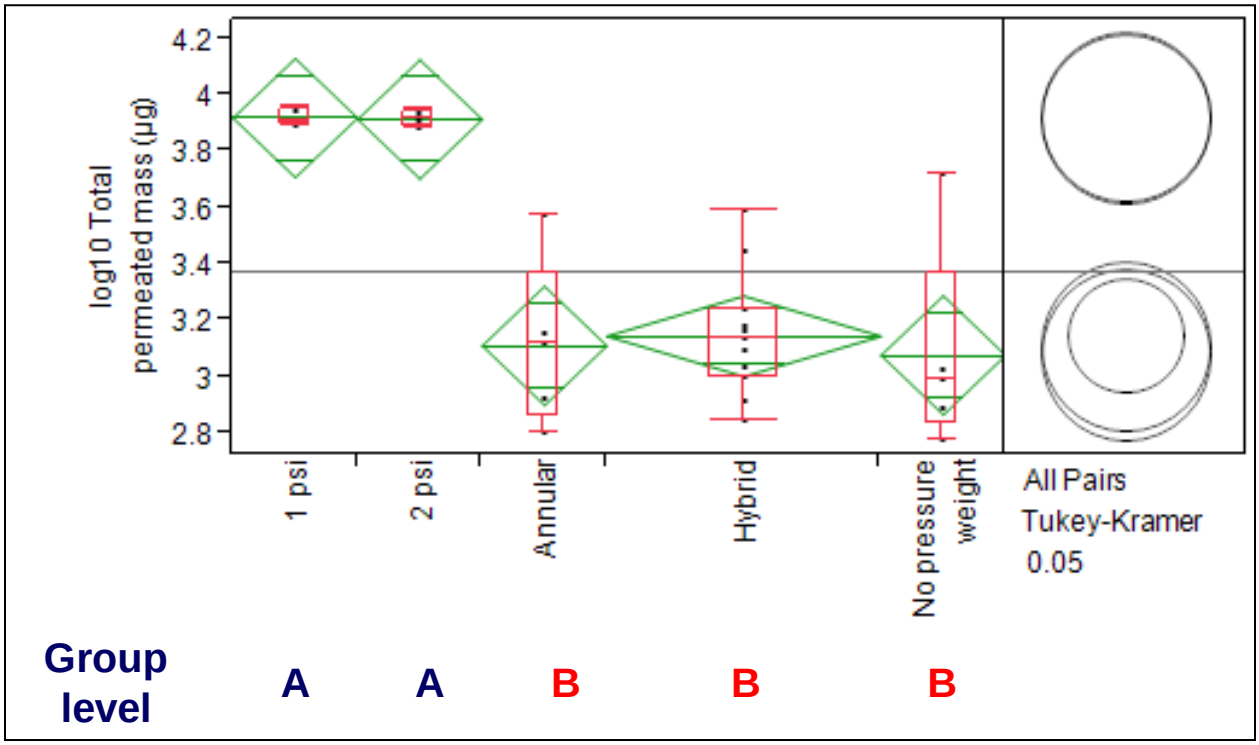

Figure 11. Tukey-Kramer analysis for various contact test configurations.

Figure 12. In the annular ring configuration, the VX-contaminated latex swelled and puckered away from the DVB pad.

7. CONCLUSIONS

A new permeation test method was developed that focuses on contact transfer. Contact transfer is a critical component for low-volatility contaminants, including VX and pesticides. These studies were designed with a number of quality controls to increase confidence in the data.

The need for a contact method was demonstrated with examples illustrative of toxicological effects. Direct contact of contaminated latex caused rapid, lethal effects, whereas with a small (1 cm) offset, zero to mild toxicological effects were observed.

Direct comparison studies of contact, hybrid, and vapor-only configurations revealed major statistical differences. In this study, when measured with contact, 83% of the initial contamination had permeated. However, only 0.5% was measured with a vapor-only configuration. Not using a contact method could grossly underestimate the potential hazard.

Results of evaluating other contact configurations, including greater and lesser contact pressures, indicated that direct pressure appears to be necessary to ensure proper contact between the PPE swatch and the sorbent layer. Pressure around the edge was insufficient, as the swatch could pucker and pull away from the sorbent layer. Furthermore, the addition of greater pressure did not increase the cumulative permeation; this indicates that the pressure did not force the contaminant through the swatch, but rather provided a mechanism for intimate contact.

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ACRONYMS AND ABBREVIATIONS

AChE	acetylcholinesterase
ALS	automatic liquid sampler
ASTM	American Society for Testing and Materials
AVLAG	Aerosol-Vapor-Liquid Assessment Group
BQL	below quantification limit
CCV	continuing calibration verification
CNS	central nervous system
CTF	Chemical Transfer Facility
DTNB	Ellman reagent, 5,5′-dithiobis-(2-nitrobenozic acid)
DTRA	Defense Threat Reduction Agency
DVB	divinyl benzene
ECBC	U.S. Army Edgewood Chemical Biological Center
GC	gas chromatography
GC-FPD	gas chromatography with flame photometric detection
HSD	honestly significantly different
IACUC	Institutional Animal Care and Use Committee
IAW	in accordance with
ID	identification number
JSTO	Joint Science and Technology Office
LD_{50}	dosage lethal to 50% of population
MRM	multiple-reaction monitoring
MS	mass spectrometer
m/z	mass-to-charge ratio
PASB	Permeation and Analytical Solutions Branch
^{31}P-NMR	^{31}P-nuclear magnetic resonance
PNS	peripheral nervous system
PTFE	polytetrafluoroethylene
RSD	relative standard deviation

SD	standard deviation
SDS–HGB	sodium dodecyl sulfate–hemoglobin
SPE	solid-phase extraction
TNB^-	2-nitro-5-chlorobenzaldehyde
VX-G	VX as regenerated G-agent analog

VX


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